Claims

1. A method for determining a substrate contained in a hemoglobin-containing sample through reaction of an oxidase with the substrate and optical measurement of the produced hydrogen peroxide by use of a peroxidase and an oxidizable color producing reagent, characterized in that the hemoglobin-containing sample is treated with an anionic surfactant selected from among a polyoxyethylene alkyl ether sulfate salt, a polyoxyethylene alkylphenyl ether sulfate salt, a polyoxyethylene alkyl ether phosphate, a polyoxyethylene alkyl sulfosuccinate, a polyoxyethylene alkyl ether carboxylate salt, a polyoxyethylene alkyl ether sulfonate salt, triethanolamine lauryl sulfate, an alkyl sulfosuccinate, and an alkylphenyl ether sulfonate salt.

2. A method according to Claim 1, wherein the anionic surfactant is a polyoxyethylene alkyl ether phosphate, a polyoxyethylene alkyl ether sulfate salt, or an alkyl sulfosuccinate.

3. A method according to Claim 1 or 2, wherein the substrate is uric acid, and the oxidase is uricase.

4. A method according to Claim 1 or 2, wherein the substrate is a fructosyl amino acid or a fructosyl peptide, and the oxidase is a fructosyl amino acid oxidase or a fructosyl peptide oxidase.

5. A reagent for determining a substrate contained in a hemoglobin-containing sample, containing (A) an anionic surfactant selected from among a polyoxyethylene alkyl ether

sulfate salt, a polyoxyethylene alkylphenyl ether sulfate salt, a polyoxyethylene alkyl ether phosphate, a polyoxyethylene alkyl sulfosuccinate, a polyoxyethylene alkyl ether carboxylate salt, a polyoxyethylene alkyl ether sulfonate salt, triethanolamine lauryl sulfate, an alkyl sulfosuccinate, and an alkylphenyl ether sulfonate salt, (B) an oxidase which acts on the substrate to produce hydrogen peroxide, and (C) a peroxidase and an oxidizable color producing reagent.

6. A method for determining glycated protein concentration, or glycated peptide or glycated amino acid concentration, or ratio of glycated peptide or glycated amino acid concentration to glycated protein concentration, characterized by employing at least (1) a surfactant, (2) a protease which acts on glycated protein to release fructosyl peptide, and (3) an enzyme which acts on fructosyl peptide to produce hydrogen peroxide.

7. A method according to claim 6, wherein the surfactant is a nonionic surfactant and/or an anionic surfactant selected from among a polyoxyethylene derivative, a polyoxyethylene alkyl ether sulfate salt, a polyoxyethylene alkyl ether phosphate ester, triethanolamine lauryl sulfate, an alkyl sulfosuccinate, and an alkylphenyl ether sulfonate.

8. A method according to claim 7, wherein the phosphate ester is a monoester or a diester of a polyoxyethylene alkyl ether phosphate, or a mixture of the monoester and diester.

9. A method according to any one of claims 6 to 8,

wherein the fructosyl peptide released through reaction of a protease with a glycated protein or a glycated peptide is fructosyl valylhistidine.

10. A method according to any one of claims 6 to 9, wherein the protease is derived from a microorganism belonging to the genus *Bacillus*, *Aspergillus*, or *Streptomyces* or their recombinant products, and releases at least fructosyl valylhistidine when one or more species of the protease are caused to act on glycated protein.

11. A method according to any one of claims 6 to 10, wherein at least fructosyl valylhistidine is a substrate of the enzyme which produces hydrogen peroxide through reaction with a fructosyl peptide.

12. A method according to any one of claims 6 to 11, wherein the glycated protein is hemoglobin A1c.

13. A method for determining hemoglobin concentration, hemoglobin A1c concentration, or ratio of hemoglobin A1c concentration to hemoglobin concentration of a hemoglobin-containing sample, characterized by comprising at least determining hemoglobin contained in the sample which has been treated with a surfactant, reacting a protease which releases fructosyl valylhistidine with the reaction mixture employed in the hemoglobin measurement, and determining hemoglobin A1c concentration of the mixture.

14. A method for determining hemoglobin concentration, hemoglobin A1c concentration, or ratio of hemoglobin A1c concentration to hemoglobin concentration of a hemoglobin-

containing sample, characterized by comprising a step of mixing the sample containing hemocytes with a reaction mixture containing a surfactant to thereby release hemoglobin from the hemocytes through hemolysis, a step of diluting the resultant mixture and optically determining hemoglobin concentration of the diluted mixture, a step of causing a protease to act on hemoglobin contained in the mixture to thereby release at least fructosyl valylhistidine, a step of causing an enzyme which reacts with fructosyl valylhistidine to thereby produce hydrogen peroxide to act at least on the released fructosyl valylhistidine, a step of reacting the produced hydrogen peroxide with a peroxidase and an oxidizable color producing reagent, a step of measuring change in absorbance of the colored compound to thereby determine the hemoglobin A1c concentration, and a step of calculating ratio of hemoglobin A1c concentration to hemoglobin concentration of the sample by use of the hemoglobin concentration and the hemoglobin A1c concentration.

15. A method according to claim 14, wherein the substrate contained in a sample is fructosyl valylhistidine or a biological component other than fructosyl valylhistidine, the component being derived from the sample which is added to the reaction mixture and producing fructosyl valylhistidine through reaction with a protease, and the enzyme which produce hydrogen peroxide is a fructosyl peptide oxidase.

16. A method for pretreating a sample in measurement of hemoglobin concentration, hemoglobin A1c concentration, or

ratio of hemoglobin A1c concentration to hemoglobin concentration, characterized by employing a nonionic surfactant and/or an anionic surfactant selected from among a polyoxyethylene derivative, a polyoxyethylene alkyl ether sulfate salt, a polyoxyethylene alkyl ether phosphate ester, triethanolamine lauryl sulfate, an alkyl sulfosuccinate, and an alkylphenyl ether sulfonate.

17. A method for determining hemoglobin concentration of a sample including the steps of releasing hemoglobin from hemocytes through hemolysis and determining hemoglobin concentration, characterized by employing at least a nonionic surfactant and/or an anionic surfactant selected from among a polyoxyethylene derivative, a polyoxyethylene alkyl ether sulfate salt, a polyoxyethylene alkyl ether phosphate ester, triethanolamine lauryl sulfate, an alkyl sulfosuccinate, and an alkylphenyl ether sulfonate.

18. A method for determining ratio of hemoglobin A1c concentration to hemoglobin concentration of a sample by means of a biochemical automatic analyzer, characterized in that, in setting operation conditions of the biochemical automatic analyzer, (1) operation conditions for measurement of hemoglobin concentration and for measurement of hemoglobin A1c concentration are individually set, (2) a reagent for determining hemoglobin concentration can also be used as a component of a reagent for determining hemoglobin A1c concentration, (3) a sample for hemoglobin concentration measurement and a sample for hemoglobin A1c concentration

measurement can be used in common, and (4) the same wavelength can be employed in hemoglobin concentration measurement and hemoglobin A1c concentration measurement.